INTRA- AND INTERSPECIFIC CHROMOSOME VARIATION IN THE LIZARD ANOLIS CRISTATELLUS AND ITS CLOSEST RELATIVES

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INTRODUCTION

Widespread on the Puerto Rican bank (Puerto Rico, its offshore keys, and the Virgin Islands, excluding St. Croix) is the common lizard *Anolis cristatellus*. Data on its ecological distribution have been summarized by Rand (1964). The last taxonomic revision (Grant, 1931) recognized three subspecies: *A. c. cristatellus,* found virtually throughout Puerto Rico, *A. c. wileyae*[4] from the islands east of Puerto Rico, including both the adjacent keys and the Virgin Islands, and *A. c. cooki* from the extremely dry, desert-like southwestern part of Puerto Rico. Thomas (1966) used the combination "*Anolis cooki*" in a discussion of the endemicity of the herpetofauna of southwestern Puerto Rico but did not justify his usage. *Anolis monensis* on the island of Mona to the west of Puerto Rico has been considered a separate species, but it is very close to *cristatellus* and its taxonomic status needs reassessment. Unfortunately, specimens of this species were unobtainable for chromosomal analysis.

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[4] Although described by Grant as *Anolis cristatellus wileyi,* the name must be changed to take the feminine ending, as the subspecies was named after Grace Olive Wiley (Grant, 1931). (See articles 31 and 32, International code of Zoological Nomenclature.)

Evidence that *cooki* is a separate species was obtained by Albert Schwartz and David Leber in 1961 when they collected two kinds of *cristatellus*-like anoles on the islet of Caja de Muertos off the south coast of Puerto Rico. Later, Thomas observed that where *cooki* is sympatric with *cristatellus*, along coastal areas of southwest Puerto Rico, it appears to split the structural niche (terminology from Rand, 1964) by perching lower than *cristatellus*, often on rocks or on the ground rather than in trees. Although morphologically very similar to *cristatellus*, *cooki* has larger dorsal scales (Fig. 1). It also has lower 4th toe lamellar counts and keeled ventral scales, and in coloration it is typically paler than *cristatellus* and has a redder dewlap. The morphological differences are most valid when contrasted with sympatric *cristatellus*. For these reasons and for reasons presented below, we consider *cooki* a separate species. Independently, E. E. Williams has come to similar conclusions by
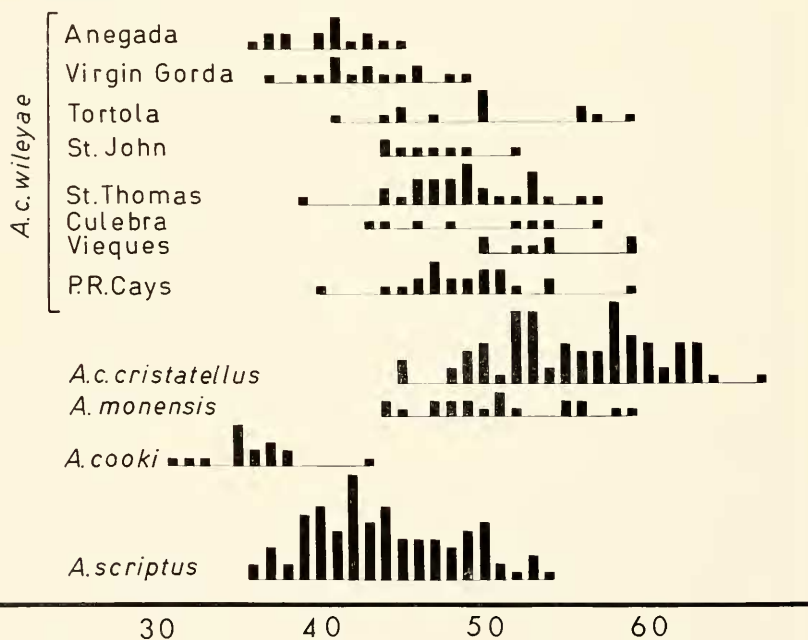

Figure 1. Dorsal scale counts (taken dorsolaterally in the standard distance) for *Anolis cristatellus* and its closest relatives. The samples for Tortola and St. Thomas include specimens from satellite islets; specimens examined are in the Albert Schwartz Field Series and the Museum of Comparative Zoology. The smallest vertical units represent single individuals.

comparison of paratypes of *cooki* with *cristatellus* from nearby localities.

The final species to be considered is *A. scriptus,* from the southern Bahamas. This species had been synonymized with *cristatellus* by Barbour (1914), but Rand (1962) showed that there were minor scale differences. He considered *scriptus* a valid species although close to *cristatellus.* Of the four described subspecies, all were recognized by Rand, and two were available for chromosomal analysis.

The forms discussed here are distinguishable by differences in coloration, especially dewlap color; their external morphology, although not always diagnostic, shows interesting regional variation. The variation in dorsal scale counts of *cristatellus* (Fig. 1) may be taken as a paradigm of trends shown by other morphological characters in this species. Tail crest height, degree of ventral scale keeling, and counts of 4th toe lamellae and loreal scales show the same pattern of variation, although the trends are generally less pronounced than are those shown by the dorsal scale counts. *A. cooki* and *A. monensis* also follow this trend in having low loreal and lamellar counts *(cooki* is extreme in its low number of lamellae). It is of interest that the *cooki* and eastern populations of *wileyae* agree with one another in having large dorsal scales, few 4th toe lamellae, few loreals, and relatively pronounced ventral keeling. This may be part of a similar adaptive constellation of characters; it should be noted that all of the small island forms *(sensu lato:* including *cooki)* show tendencies towards enlarged dorsal scales in comparison with nominate *cristatellus.*

All of these lizards belong to the *cristatellus* series, following the classification of *Anolis* into species series by Etheridge (1960). Also included in this series are four other Puerto Rican species: *krugi, pulchellus, poncensis* (grass anoles), and *gundlachi,* a species which resembles *cristatellus* and appears to replace it ecologically at high altitudes (Rand, 1964); and three species of Hispaniolan anoles, *cybotes, whitemani,* and *shrevei,* which appear very closely related *inter se* and seem to resemble most closely *A. cristatellus.*

Considerable data have already been presented on the chromosomes of *Anolis.* A karyotype consisting of six pairs of metacentric macrochromosomes and twelve pairs of microchromosomes (2n=36) is found in numerous species of *Anolis* and other iguanid lizards. This formula is considered primitive for the family Iguanidae (Gorman, Atkins, and Holzinger, 1967). Lizards with this karyotype lack obvious sex chromosomal heteromorphism, as illustrated by *A. cybotes,* a Hispaniolan member of the *cristatellus*

series (Gorman and Atkins, 1966, fig. 1 d-f). Other anoles are characterized by a reduced diploid number, and a complex sex chromosome system in which males are X_1X_2Y and females $X_1X_1X_2X_2$ (Gorman and Atkins, 1966). The karyotypes of the four Puerto Rican *cristatellus* group anoles that have already been reported on *(pulchellus, poncensis, krugi,* and *gundlachi)* are of this sort. The karyotypic difference between the Puerto Rican and His- paniolan members of the *cristatellus* series and its phylogenetic implications are discussed elsewhere (Gorman and Atkins, in press) and will not be repeated here.

MATERIALS AND METHODS

Chromosome spreads were obtained by direct preparation of gonads or by whole blood tissue culture using a modification of the technique of Moorhead, *et al.* (1960). Techniques were outlined in Gorman and Atkins (1966). Chromosome data were obtained from 19 *A. c. cristatellus* from Puerto Rico, 14 *A. c. wileyae* from the islands east of Puerto Rico, 6 *A. cooki* from southwestern Puerto Rico, 3 *A. scriptus leucophaeus* from Great Inagua, and one *A. scriptus mariguanae* from Mayaguana. Figures 2-4 show the localities sampled in this study. For comparative purposes, data are presented on two additional Puerto Rican members of the *cristatellus* series.

Figure 2. Localities on Puerto Rico sampled for *A. c. cristatellus* (circles) and *A. cooki* (squares). The number within each symbol is the number of specimens sampled for that locality.
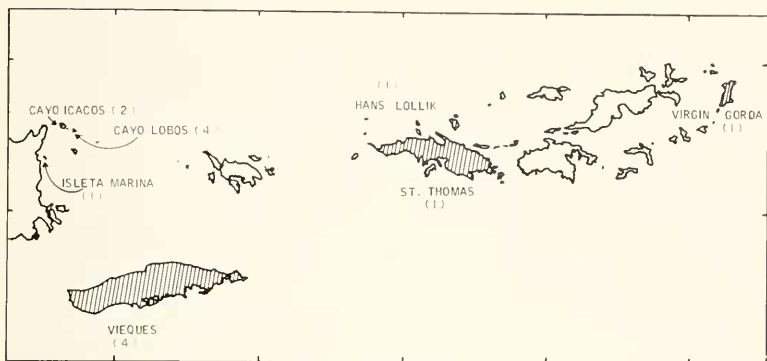
Figure 3. Map of the eastern portion of the Puerto Rico bank; islands where specimens were sampled are named. Number in parentheses is the number of specimens from each locality.
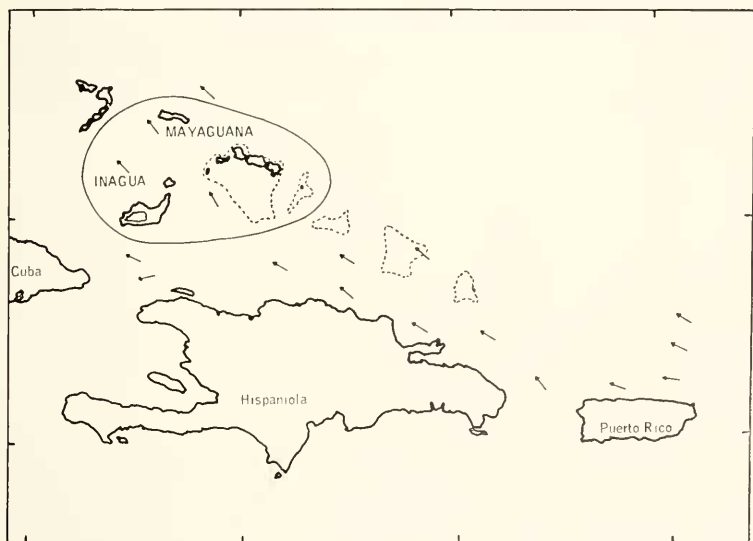

Figure 4. Map of the southern Bahamas and eastern Greater Antilles. The area enclosed in solid black represents the distribution of *A. scriptus*. Inagua and Mayaguana are the localities sampled. Dotted areas represent submerged island banks. Arrows show general trend of the currents.

RESULTS

Male meiosis. The four species in the *cristatellus* group previously studied *(gundlachi, pulchellus, poncensis, krugi,* see Gorman and Atkins, in press) are all very similar to one another. In diakinesis there are six large bivalents, two intermediate-sized bivalents, five small bivalents (13 autosomal bivalents in all), and a sex trivalent. Figure 5a shows this in *A. gundlachi.* Among the forms reported on here, only *A. cooki* has 13 autosomal bivalents and a sex trivalent. *A. cooki* differs from *gundlachi* and *pulchellus* only in the detail that the smallest bivalents do not break sharply in size when compared with the intermediates (see Figure 5b).

All *A. c. cristatellus* and both the *scriptus* subspecies differ from *cooki* and the other members of the *cristatellus* series by having one fewer autosomal bivalent. Again there are six macrobivalents; however, there are only six smaller ones, which apparently can be divided into two classes of three intermediate-sized and three small, but certainly with gradation (Figs. 5c, d).

It is among the offshore populations of *A. cristatellus wileyae* that we have made the most unusual finding. Twelve of the animals sampled appeared identical to the Puerto Rican *cristatellus* and Bahaman *scriptus,* but two others differ in having a moderate-sized body that might be a univalent. One of these animals is from the small key Cayo Lobos, the second is from Vieques. Three of the four males sampled from each of the two islands had normal meiosis (Fig. 6).

Mitosis. The mitotic metaphase of males of *cooki* is characterized by 29 chromosomes. There are six pairs of metacentric macrochromosomes and 17 smaller elements. Resolution of these elements is difficult, but there appear to be three pairs of small metacentric chromosomes, four dot-like pairs (presumably acrocentric), and three unpaired chromosomes, the largest of which is clearly metacentric and is presumably the Y (Fig. 7b). Females have 30 chromosomes and lack the unpaired metacentric. The male karyotype is similar to *pulchellus* (Fig. 7a), but again, as in meiosis, the break between size classes of chromosomes is greater in *pulchellus*. There are six pairs of metacentric macrochromosomes, two intermediate pairs of metacentric chromosomes, five pairs of microchromosomes, and, in the male, three unpaired chromosomes (2n=29).

A. scriptus and *A. c. cristatellus* have male diploid numbers of 27; female *scriptus* have 2n=28 (female *cristatellus* were not studied). The male diploid number of 27 is also found in *A. c. wileyae* (one exception will be discussed below).
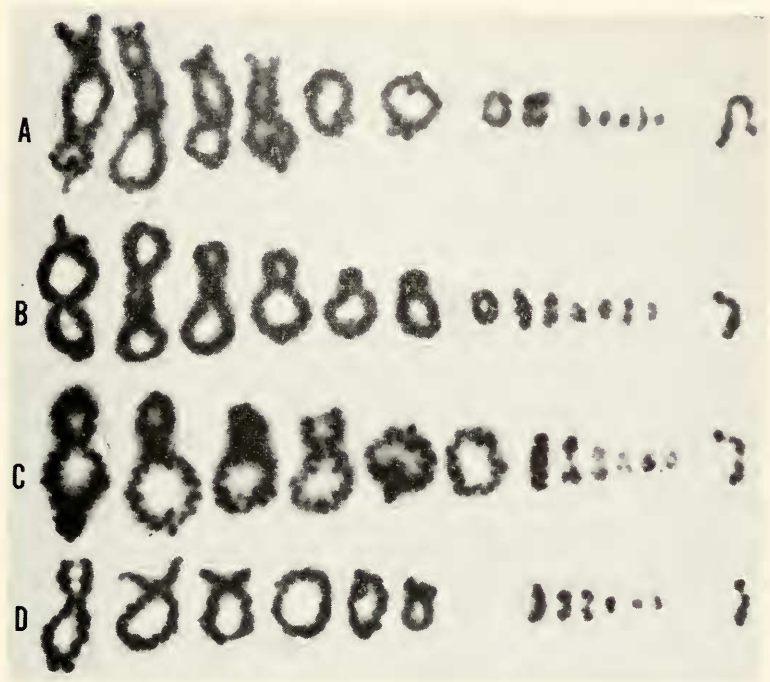
Figure 5. Meiosis in *cristatellus* group anoles. Giemsa stain.

A. *A. gundlachi*. There are six large bivalents, two of intermediate size, and five distinctly smaller bivalents; a sex trivalent is on the far right.

B. *A. cooki*. There are six large bivalents, seven ranging from intermediate to small size with no sharp break between them; and a sex trivalent.

C. *A. c. cristatellus*. There are six large bivalents, and only six ranging from intermediate to small size with no sharp break between them; and a sex trivalent.

D. *A. scriptus leucophaeus*. Quite similar to *cristatellus* directly above.

In details of karyotype, *cristatellus* and *scriptus* are very similar. There are six pairs of metacentric macrochromosomes and six autosomal pairs of small chromosomes, of which the largest three pairs are also metacentric. Males have three unpaired sex chromosomes (Figs. 7c, d).

The one exceptional animal was the *A. c. wileyae* from Vieques which had the extra body (presumed univalent) in meiosis (no mitotic divisions were seen in the specimen from Cayo Lobos that
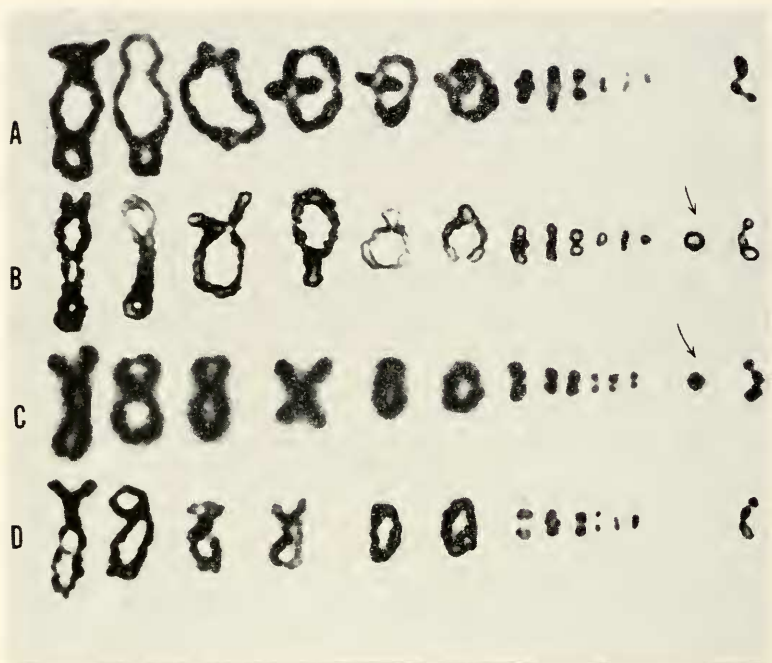
Figure 6. Meiosis in *A. cristatellus wileyae*. Giemsa stain.

A and B. Two different males from the island of Vieques. Male A is identical to typical *cristatellus*, male B has a dark staining supernumerary body (arrow).

C. A male from Cayo Lobos that also shows a dark staining supernumerary body (arrow).

D. A male from St. Thomas, Virgin Islands, which has the typical *cristatellus* complement.

had the same meiosis). Only 10 mitotic spreads could be counted from the testis of this animal, of which 4 had 27 chromosomes (the same as other *cristatellus*) and 6 had 28, or one extra chromosome. The extra chromosome appears to be a microchromosome. Possibly, the animal is a 27/28 mosaic. However, interpretation is difficult. Because of the small size of the microchromosomes, it is easy to have one masked by a large arm, and it is indeed possible that the real diploid number is 28. Ten counts are far too few to establish the karyotype with certainty. It should be emphasized that the two chromosomally aberrant *wileyae* did not differ morphologically from the other lizards sampled.
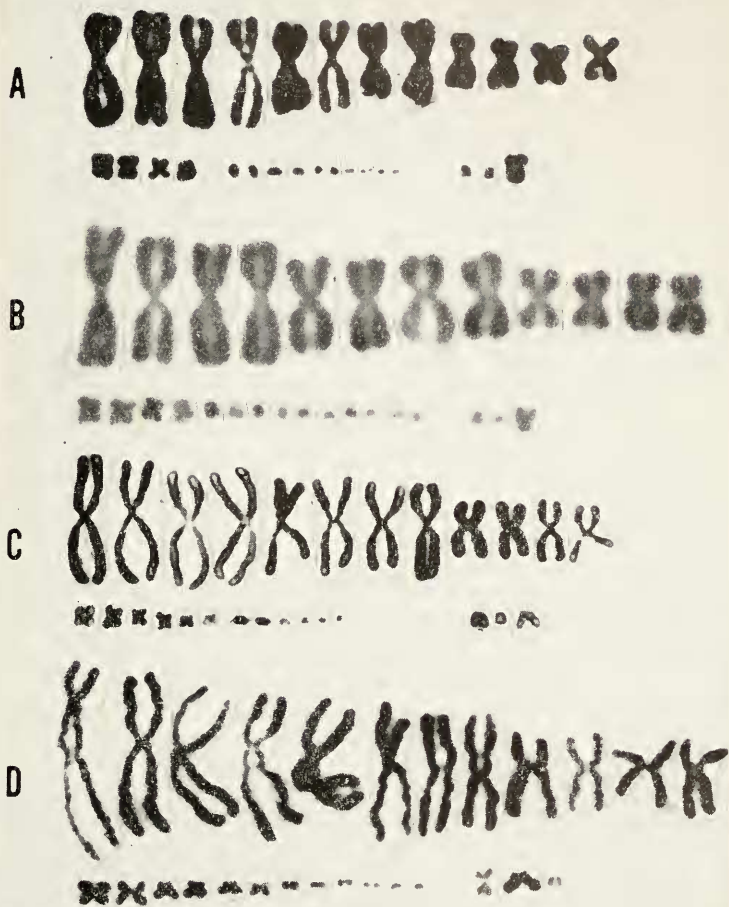
Figure 7. Mitosis in males of *cristatellus* group anoles. Giemsa stain.

A. *Anolis pulchellus* (2n=29). There are six pairs of metacentric macrochromosomes (top row), two pairs of small metacentric chromosomes (far left of second row), a sharp break between these and the five pairs of microchromosomes, and three unpaired sex chromosomes.

B. *A. cooki* (2n=29). Macrochromosomes as above (top row). Pairs seven and eight, small metacentrics, are as in *pulchellus*, but there is no sharp break between pair eight and pair nine. There are again three unpaired sex chromosomes.

C. *A. cristatellus wileyae* (2n=27). Macrochromosomes as in the above species. Microchromosomes most similar to *cooki*, but one pair fewer. Sex chromosomes similar.

D. *A. scriptus leucophaeus* (2n=27). Resembles *cristatellus*.

DISCUSSION

The chromosomal data support the hypothesis that *cooki* is a species distinct from *cristatellus*. This is in accord with observations made by Thomas in the field. There are two alternative explanations which may account for the observed chromosomal complements of these two species and their present-day distribution:

1. *A. cristatellus* has the lowest diploid number in its species group. Reduction in chromosome number is *usually* the derived condition and, if this be so in this case, *cristatellus* is a derived form. Chromosomal heterozygotes experience meiotic difficulties, leading to reduced fitness; the resultant structural mutants would be swamped in large populations. Chromosome structural changes, therefore, are probably established in small isolated populations. Thus it is logical to assume that the stock leading to *cristatellus* evolved in the limited population of a small island off Puerto Rico. Once chromosome loss had been established, *cristatellus* reinvaded the main island, successfully filling the lowland arboreal niche throughout Puerto Rico.

A. cooki has a very restricted range (southwestern Puerto Rico) and is specialized for a very dry rocky area. One hypothesis accounting for its origin is that it may have been isolated from its ancestral stock on an insular region corresponding to the present coastal hills of southwestern Puerto Rico. This stock, quite possibly the same one that gave rise to *cristatellus* (for the two species are remarkably similar in appearance), might have had the primitive karyotype for the species group.

The diploid number of *cooki* (29) is the same as that of *gundlachi* and the grass anoles of the *cristatellus* series, but there are some differences in the details of karyotype. The six pairs of macrochromosomes are comparable in all the forms. In *cooki* there is then a gradation from pairs 7 to 13, of which the largest three are metacentric (see Fig. 7), while in the grass anoles and *gundlachi* pairs 7 and 8 are medium-sized metacentrics which break sharply in size with the smaller pairs (9-13).

With respect to the microchromosomes, *cristatellus* resembles *cooki* in that pairs 7, 8, and 9 are all metacentric and grade in size into the smaller elements. The major difference, of course, is that *cristatellus* has one fewer pair of microchromosomes.

Hence we might postulate that *cooki,* although morphologically specialized, retains the primitive karyotype. *A. cristatellus,* then, is similar but has suffered chromosome loss, while *A. gundlachi* and the three grass anole species are the products of a single

radiation in which a new karyotype was established by reciprocal translocations from a species with the primitive *(cooki*-like) karyo-type.

2. An alternative hypothesis would consider the *gundlachi*-type of karyotype primitive for the species group. *A. cristatellus* would again have been derived from a $2n=29$ ancestor by chromosome loss, followed by minor rearrangements. The apparently closely related *A. cooki* might then have been directly derived from *cris-tatellus* by centric fission, which secondarily raised the male diploid number back to 29.

Karyotypic data, in any case, show that *gundlachi* is closer to the grass anoles than it is to *cristatellus*. This is not obvious from external morphology but is in accord with serum protein data (Maldonado and Ortiz, 1966) and osteological data (Etheridge, 1960). In karyotype, *gundlachi* is virtually indistinguishable from the grass anoles.

It is a further point of interest that the chromosomal evidence confirms the close relationship of *scriptus* and *cristatellus*. No other anoles of the 70 species and subspecies that we have studied have a male diploid number of 27, and even details of karyotype are identical.

From a glance at a map, one might expect that the southern Bahamas would be colonized from Hispaniola rather than Puerto Rico. However, as E. E. Williams pointed out to us, the general current flow is northwesterly, and there are several barely sub-merged banks between Puerto Rico and the southern Bahamas, banks which may have served as stepping stones for colonization. It is therefore not surprising to find a Puerto Rican derivative on these islands (see Fig. 4). The widespread Hispaniolan member of the *cristatellus* group, *A. cybotes*, has a diploid number of 36 in both males and females with no heteromorphism and could not possibly be the direct ancestor of *scriptus*.

The finding of apparent supernumerary chromosomes in two island populations of *A. c. wileyae* is of cytological interest, but lack of data at the present time on the extent of this variation within the populations and on the behavior of the supernumerary precludes discussion.

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